The Use of Fibrinolytic Agents

Present Status in the Treatment of Cardiovascular Disease

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• **Fibrinolysins are involved in the dissolution of clots. They are still in the experimental stage and are not yet ready for use by practicing physicians. The materials available for clinical use have undesirable serious side effects. However, the future for fibrinolysins in clinical practice has promise.**

CLOTTING IS A complex mechanism resulting in a great variety of changes. There are means which can either retard or prevent clotting and others which can dissolve a thrombus once it is formed. Knowledge of thrombus lysis is still fragmentary, but it has been sufficiently intriguing to have set into motion a series of studies on the mechanisms involved. These studies have been based on analyses of experiments conducted both *in vitro* and *in vivo*.

Unfortunately the attitude that what applies in such experiments can be transferred in its entirety to man has led to the premature clinical availability of fibrinolytic agents. This has resulted in a rash of clinical reports appearing like an epidemic. It should be stated categorically that fibrinolytic agents have no established place today in clinical practice; they are to be considered investigative drugs only.

Unexpected benefits may come out of activities with new drugs and this is true in the case of the fibrinolytic studies. Sherry, who (with Astrup, the Ambruses, Cliffton, and Johnson, among many others) has led recently in this field of study, found a chemical, epsilon-amino caproic acid, among the agents which inhibit activation of plasmin (the name given to the fibrinolytic agent in the blood). This inhibitor has been found to be useful in lessening the oozing of blood which occurs after prostatectomy and in uterine bleeding. This example of serendipity is at the moment of far more practical value than that derived from any clinically available fibrinolytic agent. Disorders of the fibrinolytic mechanism will become more clearly defined as our knowledge advances. Some of these disorders will be found to lead to bleeding tendencies, others to the accelerated deposition of fibrin thrombi. There has been some discussion recently concerning the possible role of such fibrin thrombi in the production of atherosclerosis. While this mechanism may be of importance in the progression of atherosclerosis and in the development of some of its sequelae, it is not primarily involved in the formation of the basic atheroma.

The fibrinolytic system must be kept in mind in any consideration of thromboembolism. The various components of the fibrinolytic system exist in the blood stream and in the tissues in amounts sufficient to influence the formation and dissolution of thrombi. Furthermore, it is clearly established that the amounts of these components and their interaction can be readily modified. Thus, it is considered that there is a constant tendency for thrombosis to occur because of minor trauma and stasis to which the entire vascular bed is subjected. If this is so, then fibrinolysis of these thrombi must be constantly going on to account for the infrequency of such thrombi. That such lysis may be constantly occurring is evidenced by the fact that large pulmonary emboli which do not cause the patient's death dissolve completely in many instances. Furthermore, in dogs, sizable, well formed clots placed in the portal vein have been found to dissolve. Such dissolution of thrombi may be of importance in the recanalization of occlusive thrombi and in wound healing. Normal blood contains a pro-enzyme *plasminogen* (perhaps derived from the eosinophiles) which when activated becomes the enzyme *plasmin*. This enzyme is capable of hydrolyzing the internal peptide bonds of the protein fibrin as well as those of some other proteins. Plasmin is contained in euglobin (Fraction III of Cohn). Even the purest plasmin is said to contain about 30 per cent impurities. It appears to act on the peptide bonds of arginyl and lysyl residues. Sherry estimated that the molecular weight of plasminogen is 143,000 and of plasmin 108,000. Both plasmin and plasminogen have an affinity for fibrin and fibrinogen. This is an advantage for their specialized fibrinolytic activity.

The conversion of plasminogen to plasmin requires an activator. This conversion can occur, *in vitro*, either spontaneously or with certain agents such as chloroform, by precipitation of the globulin fraction of the blood. It can also be activated by tissue kinases, by urokinase obtained from the

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urine and by trypsin. Kinases are present also in milk and tears. The conversion of plasminogen to plasmin can be activated by plasmin itself as an autocatalytic reaction. Bacterial kinases, notably streptokinase and staphylokinase, are effective. Tissue kinases are tightly bound to structural proteins but at least part of them can be readily extracted. Lung and brain appear to have the highest concentration. In the aorta, the tissue kinase is highly concentrated in the adventia with a much smaller amount in the media and intima. In the veins the intima has a greater concentration than in the arteries.

There has been little progress in purifying tissue kinase but considerable work has been done on the preparation of urokinase in stable form. Streptokinase is an extracellular bacterial protein produced by actively growing hemolytic streptococci of the Lancefield group A, human C and G. These streptokinases are impure but fairly specific for human plasminogen and may activate a preactivator present in human plasma.

EVIDENCE has accumulated that both plasminogen and plasmin have inhibitors. Much more is known about the latter than the former. Epsilon-amino caproic acid is such an inhibitor. Ratnoff concluded that human serum contains at least three different inhibitors. Inhibitors are also present in tissues. It would appear that fibrin has a greater affinity for plasmin than do the plasmin inhibitors, and the Ambruses have suggested that slow infusion of plasmin into the blood stream may permit its combination with one of the plasmin inhibitors, allowing plasmin to be released readily for combination with fibrin whenever fibrin is encountered. Such antiplasmin-plasmin combinations, however, appear to prevent the proteolytic action of plasmin upon other proteins which may be encountered when plasmin is injected more rapidly. Recently the Ambruses showed that human fibrin clots introduced into the circulating blood of dogs after an infusion of human plasmin will exhibit *in vitro* lysis on later incubation even though no "free" plasmin could be demonstrated in the dogs' blood at the time that the clot was removed. Furthermore, they showed that plasmin-antiplasmin complexes formed *in vitro* were as effective as the same amount of plasmin alone in dissolving the fibrin clot *in vivo*.

The avidity of fibrin for plasmin is evidenced by the fact that it is difficult to prepare fibrin entirely free of plasminogen. Furthermore, the plasminogen content of exudates and transudates have been found to run parallel to their fibrin content. Plasmin when adsorbed to fibrin is seemingly protected from circulating antiplasmins.

NUMEROUS METHODS have been devised to assay plasminogen, plasmin, kinases and antiplasmin in biological fluids and therapeutic agents. Early methods employed clots formed by adding thrombin or calcium to dilute citrated plasma. These methods are only roughly quantitative and do not distinguish plasminogen-kinase-plasmin effects from those of plasmin-antiplasmin. An improvement is the use of euglobin fraction, which gets rid of the interference by antiplasmin. In these euglobin fractions, the effect is judged on exogenous or endogenous fibrin clots, casein substrates or fibrin plates. It is an easy way of assaying plasmin or plasminogen but care must be taken to avoid contamination of plasminogen attached to the thrombin or exogenous fibrinogen. Besides casein, synthetic esters of arginine and lysine have been used to measure plasmin activity.

When using fibrin plates, one can distinguish between plasmin and plasminogen simply by comparing heated and unheated plates. Since heat destroys plasminogen, one can use this method to measure plasmin and kinase.

Another method of assay is to produce a clot in a vein by means of I^{131}-labeled, plasminogen-free fibrinogen, the rate of lysis being measured by the reduction of radioactivity in the labeled clot. Isotopically tagged clot particles have also been used as emboli for this purpose. *In vivo* methods permit a measurement not only of lysis but also of side effects such as pyrogenic reactions. In these *in vivo* methods, escape of the clots from the site being measured to the general circulation will give a false result. The *in vivo* method is useful in measuring the persistence of lytic activity locally in the clot after that contained in the plasma has disappeared because of the fixation of the lytic material by the fibrin of the clot.

The value of these *in vivo* clots or *in vitro* methods is limited by the difference of the materials or clots that are used from the thrombi encountered clinically. The latter quickly lose their ability to be lysed by the plasmin system so that the material must be introduced within three to six hours or, at the latest, within 24 hours.

Besides this, the administered lytic agent must get to the thrombus. There is disagreement as to the most effective way of doing this. One view is that held by Sherry, that the best way is to use a kinase. The kinase activates the plasminogen associated with the fibrin in the thrombus. Because of the affinity of fibrin for plasmin, this prevents neutralization by the ever present plasma antiplasmins. Unfortunately, the kinases available today, the streptokinases, are pyrogenic and antigenic and hence not clinically suitable. Sufficient work is being

done on urokinase to suggest that a kinase with fewer side effects will be found for clinical use.

These are very practical objections. In the case of recent myocardial infarction, for example, the presence of pyrogenic effects with fever would make the early use of the kinases now available sufficiently hazardous because of the increased work of the heart so induced. And this would nullify any possible benefit to be derived from dissolving coronary thrombi. If one waited until the heart was in a better state, there would be less fibrinolysis even with the best kinase used and, the need for such a procedure would have become less urgent since compensatory mechanisms already present would have come into play to permit the heart to survive.

ANOTHER METHOD of inducing fibrinolysis is to use plasmin itself. If plasmin is given in high concentrations hypofibrinogemia or afibrinogemia may result. If plasmin is given relatively slowly it will have little effect on plasma proteins other than fibrin because of the greater affinity of fibrin for plasmin. In slow infusion, the plasmin is locally and rapidly combined with one of the antiplasmins in the blood. This prevents the plasmin so held from acting on other proteins but permits fibrin to pick it up readily from the loose plasmin-antiplasmin combination. This attractive concept, advanced especially by the Ambruses, is considered by many investigators to be unproved. However, the fact that plasmin does not have the side effects of presently available kinases warrants further studies.

With this background we are prepared to consider the present fibrinolysis in clinical therapeutics.

Most clinical studies have not been adequately controlled. The methods employed to judge clinical results, namely, electrocardiographic evolution, coronary artery angiography and assay of clinical status, are too inaccurate for adequate measurement of effective fibrinolysis clinically.

Not all occlusions are thrombotic. In the case of the coronary vessels, occlusion may be caused by atheromatous material. Not all thrombi have a uniform distribution of fibrin. It has been shown that in naturally occurring thrombi the head of the thrombus is formed by platelets and leukocytes with little associated fibrin. There are white and red thrombi clinically. Arterial thrombi differ from venous thrombi. As they grow older, thrombi become progressively more resistant to thrombolytic agents. After three days no fibrinolytic effect is demonstrable. Actually, unless therapy is instituted in a matter of hours little fibrinolysis can be expected.

The two products on the market, Actase® and Thrombolysin®, are both antigenic. They are mixtures of streptokinase, plasmin and other material. The kinases they contain may cause fever, shock and hypoxia. They should not be used clinically on a routine basis.

Fibrinolysins are still in the experimental stage and should not be used in clinical practice—whether for coronary thrombosis, arterial thrombosis or venous thrombosis.

In short, at present, fibrinolysins have not been proven clinically useful. The future of fibrinolysins may be much brighter. The theory is appealing. There is need for better understanding of the mechanisms involved. More studies are needed on the action of plasmin and kinases on various types of clots and on naturally occurring thrombi, both as to kind and site and age. More uniformity is needed in assaying the lysis of clots and thrombi. Purification of plasmin for ready clinical use and of a pyrogen-free non-antigenic kinase are essential. Perhaps more attention should be paid in this connection to urokinase—and a human urokinase would be a big step forward. There is also the need of more sophisticated clinical studies with proper controls—and the discouragement of the continued publication of mere testimonials even when they include tens and hundreds of cases.

BY SUCH MEANS, it is possible that a clinically suitable preparation for the rapid lysis of venous and arterial thromboses may become available for widespread use. In the case of coronary thrombosis, I doubt that fibrinolysis will turn out to be of much use because of the usual delay, often up to several hours, after the thrombosis has occurred before the patients come under active treatment. Only (if and when) such therapy can be used over an extended period of time in impending myocardial infarction do I conceive of any possible role of this therapy in myocardial infarction.

Finally, may I suggest that fibrinolysins may prove to be of value in hyaline membrane disease of newborn infants. Here experimental evidence suggests that the basis of the disease appears to be fibrin deposition. This may be due to plasmin lack in the lung resulting from the extraordinary release of antiplasmin by the placenta. It would make sense, if the facts are as stated, to attempt the employment of fibrinolysins to dissolve the hyaline membrane and relieve the baby's disability. Initial studies, however, have so far been disappointing.

In addition, I would suggest that thrombi disintegrate within blood vessels by means other than fibrinolysis. An attempt needs to be made to explore these processes intensively. Who knows but that the practical solution to thrombolysis may reside in some of these still mysterious mechanisms rather than in fibrinolysis.

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